urinary levels of collagen type II C-telopeptide fragments (uCTX-II) were measured by the CartiLaps ELISA assay. uCTX-II was corrected by urinary creatinine levels as assessed by a standard colorimetric method. In order to achieve approximately normal distribution, log(uCTX-II) was used as the marker.

At BL, the distribution of KL scores was (145,88,30,24,1) for KL 0-4. Among the BL healthy knees (KL 0), 101 were non-progressors at FU and 25 were progressors (KL $>$ 0) at FU.

Results: The mean total cartilage volume at BL was quantified to 6851 mm3 with scan-rescan CV of 3.6% (the intra-scan CV is zero since the method is fully automatic). The uCTX-II at BL was higher for the progressors than for the non-progressors (244 vs 173 ng/mmol, $p<0.01$, sample size 67, odds ratio 6.2, Figure 1). The cartilage loss for the low uCTX-II group (less than mean uCTX-II) at BL was lower than for the high uCTX-II group ($p=0.02$, Figure 2).

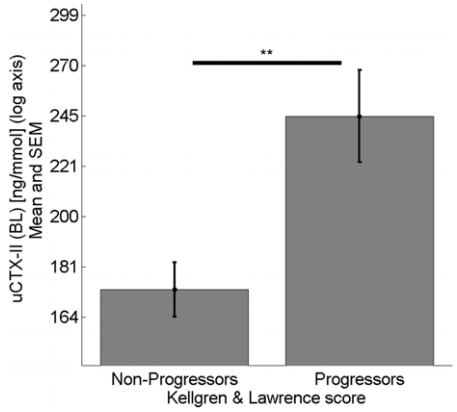

Figure 1. Increased uCTX-II at BL was a predictor of early OA progression as defined by radiographic signs (increased KL score at FU from 0 at BL).

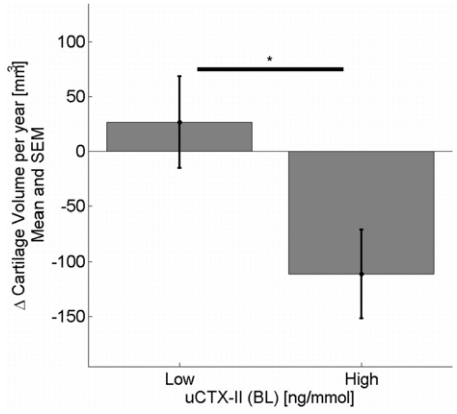

Figure 2. Increased uCTX-II at BL was a predictor of cartilage loss over 21 months.

Conclusions: Clinical trials of potential DMOADS rely on proper selection of the study population. The selection criteria must ensure that the population is likely to progress in OA level without treatment - otherwise it will be impossible to show a treatment effect for any DMOAD. The results suggested that uCTX-II is indeed a suitable prognostic marker for use in selection criteria. Elevated BL uCTX-II implies an increased risk of OA progression (progressors are 41% increased, odds ratio 6.2) - defined by either KL score or by cartilage loss. Thereby, the pair of uCTX-II and cartilage volume biomarkers seems suitable for selecting the study population and for quantifying the treatment efficacy in clinical studies.

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SYNCHROTRON FTIR ANALYSIS OF ARTICULAR CALCIUM-CONTAINING CRYSTALS

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Purpose: Sixty percent of synovial fluids from patients with severe osteoarthritis contain calcium pyrophosphate dihydrate (CPPD) or basic calcium phosphate (BCP) crystals. Problems in accurately identifying and characterizing calcium-containing crystals have slowed progress towards understanding the role of these pathologic crystals in osteoarthritis. Synchrotron FTIR (sFTIR) analysis has been useful to study mineral formation in bone. We sought to determine if it could be used in human synovial fluid and in mineralization models to identify and characterize calcium-containing crystals

Methods: Discarded synovial fluids containing native CPPD or BCP crystals were obtained in accordance with our local IRB. Biosynthetic calcium-containing crystals were generated in vitro using well characterized models of crystal formation, including a cell culture model with porcine articular chondrocytes and a model utilizing isolated porcine articular cartilage vesicles. Truly synthetic CPPD and BCP crystals were generated in our laboratory in an inorganic milieu. Crystal containing material was placed on an IR reflective slide. Crystal-containing areas were identified with light microscopy, and examined with a Thermo Fisher Continuum FTIR Microscope at the Synchrotron Radiation Center in Stoughton, WI. The FTIR spectra generated were compared with known spectra of multiple forms of pure calcium phosphate and calcium pyrophosphate crystals, as well as spectra generated with truly synthetic CPPD and BCP crystals and cartilage proteoglycans alone and in mixtures.

Results: sFTIR readily identified both CPPD and BCP crystals in synovial fluids and in articular cartilage vesicle models. Most CPPD crystals were monoclinic. CPPD crystals identical to those seen in synovial fluids were also readily identifiable in articular chondrocyte monolayers incubated with ATP. Most of the BCP crystals from synovial fluids and models were consistent with hydroxyapatite, rather than with the other calcium phosphate crystals types known to be present in BCP. Interestingly, careful examination of different regions of human or porcine material often revealed both CPPD and BCP containing areas in a single crystal aggregate. In spectra from many CPPD crystals, the peak at the 1134 cm -1 frequency region found on the standard spectrum for CPPD was absent or diminished. Addition of cartilage proteoglycans to truly synthetic CPPD crystals dampened the peak at this frequency region, much as this peak was diminished in biosynthetic or native CPPD.

Conclusions: sFTIR analysis allows for accurate identification of CPPD and BCP crystals generated in vivo or in vitro. These data support the presence of mixtures of crystals in a single region, and prove the validity of the monolayer model of CPPD crystal formation. they demonstrate crystal-proteoglycan interaction in vivo and in vitro. This novel application of sFTIR should further our understanding of the role of these crystals in osteoarthritis.